protein comprises a subsequence, at least 15 amino acids in length, which is identical to a subsequence of at least one of said isolated protein.

41 (new). The protein of claim 35 where said variant protein comprises a subsequence, at least 20 amino acids in length, which is identical to a subsequence of at least one of said isolated protein.

42 (new). The protein of claim 35 where said variant protein comprises a subsequence, at least 30 amino acids in length, which is identical to a subsequence of at least one of said isolated protein.

43 (new). An isolated protein derived from *Chlamydia pneumoniae* which

(1) is recognized by a polyclonal antibody raised against an SDS-treated, purified outer membrane complex of *Chlamydia pneumoniae*,

(2) has an apparent molecular weight, under SDS-PAGE, of 89-101 or 56-57 kDa

said protein being free of any other chlamydial protein.

44 (new). The protein of claim 43 which comprises at least two Gly-Gly-Ala-Ile motifs.

REMARKS

1. Formal Matters

1.1. Drafting Branch previously objected to the figures, as follows:

poor quality (half tone): Figs. 1-5, 9-11, 12
margin not acceptable: Fig. 8J (left margin)
line character: Figs. 6-8J
reference character: Figs. 2, 3, 5, 6-11
figure legends: Figs. 1-12

We submit herewith substitute Figs. 1-12. We note that Figs. 1-5, 9-11 and 12 are now fulltone.

1.2. The examined claims are 5, 7 and 10.

1.3. The new claims have basis as follows:

19 (90%)	P12, L22
20 (95%)	P12, L22
21 (98%)	P12, L22
22 (10+ AAs):	P10, L33
23 (15+ AAs):	P15, L32
24 (20+ As):	P15, L33
25 (30+AAs):	P15, L34
26 (PAB 150):	P5, L34-36
27 (2+ GGAI):	P14, L21
28 (3+ GGAI):	P14, L22
29 (4+ GGAI):	P14, L32
30 (FYDPI):	P15, L5
31 (2+ W):	P14, L22-23
32 (4+ W):	P15, L7
33 (400-490):	P15, L3
34 and 35:	P8, L5-20; P8, L31-P9, L3; P9, L3-28 (chamydial OM epitope); P14, L21 (2+ GGAI)
36 (3+ GGAI):	P14, L22
37 (4+ GGAI):	P14, L32
38 (FYDPI):	P15, L5
39 (10+ AAs):	P10, L33
40 (15+ AAs):	P15, L32
41 (20+ AAs):	P15, L33
42 (30+ AAs):	P15, L34
43 and 44:	see section 4

2. Prior Art Issues

Claims 5, 7 and 10 stand rejected as anticipated by Melgosa, et al. (OA §§14-16). These rejections are respectfully traversed.

The Examiner's position appears to be that since Melgosa et

al. thought there was just one protein in his 98 kDa band, that Melgosa et al. anticipated the examined claim to an "isolated protein" even though that band in fact contained a plurality of proteins.

The Examiner says that "the features upon which applicant relies (i.e., several different proteins) are not recited in the rejected claim(s). However, the claim recites an isolated protein, and it is evident that Melgosa et al. in fact failed to isolate any of the recited chlamydial proteins from the others.

Because applicants cloned and sequenced the genes encoding the 98/95 kDa C.pneumoniae COMC proteins, they are capable of producing each of these proteins free of other chlamydial proteins, even those of the same molecular weight band. For example, Omp4 was produced free of Omp 5, 6, 7, 8, 9, 10, 11, 12 and 13. (P8, L21-33).

In order to avoid any issue as to whether the proteins of Melgosa's band are "isolated", we have amended claim 5 (upon which 7 and 10 are dependent) to recite that the isolated protein of clause (i) is "free of any other chlamydial protein". Thus, if it is the protein of SEQ ID NO:2, it must be free of the proteins of SEQ ID NOs:4, 6, ...24, as well as free of any other chlamydial protein.

Since Melgosa did not produce any variant protein, or any fragments, it is not necessary to similarly qualify clauses (ii) and (iii).

3. Enablement Issues

The Examiner concedes enablement for the elected species, SEQ ID NO:2. (Presumably, if the species restriction were withdrawn, the other specifically disclosed species, SEQ ID NOs:4, 6, 8...24, would also be deemed enabled.)

However, the Examiner says that the "variants and subsequences claimed" are not enabled.

The "variants" of claim 5(ii) are required to have an amino acid sequence identity of at least 80% to at least one of the isolated proteins of (i), and to comprise at least one epitope of at least one of said isolated proteins.

The Examiner says that the specification (1) does not teach any functional variants and (2) does not discuss which positions can be altered. The examiner also points to examples of mutagenesis experiments in which a loss or shift of activity occurred as a result of a small number of mutations. These examples are simply not well known.

Claim 5 is directed to a protein per se, and hence is enabled if there is any use for the claimed proteins.

Applicants do not propose to use these proteins in order to duplicate their normal biological function in Chlamydia. Rather, they are concerned solely with their immunological activity in mammals.

Applicants teach two utilities:

- diagnosis of infections with Chlamydia pneumoniae
- vaccination against Chlamydia pneumoniae infections

(P1, L9-11; P3, L31-34).

In the first utility, the proteins would be used in the detection of antibodies against the chlamydial proteins. (P4, L1-6). For this utility, all that is necessary is that the claimed protein present an epitope which cross-reacts with one on the chlamydial surface and elicits a humoral immune response (not necessarily a protective one) in a mammal.

In the second utility, the proteins would be used as vaccines. For this utility, it is additionally necessary that the epitope cross-reacts with a chlamydial epitope which elicits a protective immune response (humoral or cellular). The balance of these remarks will focus on the first utility, since only one

utility is needed and it has the more relaxed requirements.

Claim 5 requires that both the variant protein of (ii) and the subsequence of (iii) comprise "at least one epitope of at least one of the isolated proteins". That satisfies the basic requirement for the first utility.

The question then, is not whether it would take undue experimentation to identify the residues essential to the biological activity of these proteins. Rather, it is whether it would take undue experimentation to identify epitopes of the proteins.

There are two basic methods of identifying linear epitopes. The first is to analyze the sequence for subsequences which are deemed likely, based on knowledge of epitopes generally, to be recognized by the immune system. The first algorithm of this type was the one developed by Hopp and Woods, Proc. Nat. Acad. Sci. (USA), 78:3824-8 (1981), copy enclosed, which looks for regions of high hydrophilicity. A more complex algorithm is that of Jameson and Wolf, which calculates an "antigenic index" for each subsequence. See Jameson and Wolf, CABIOS, 4(1):181-6 (1988), copy enclosed.

At P28, L13-15, and P33, L8-9 Applicants teach the use of the Wisconsin GCG package for sequence analysis.

The GCG package included several programs relevant to epitope identification, notably PepPlot, PeptideStructure and PlotStructure, and Antigenic.

PepPlot provided hydrophilicity plots using either Hopp-Woods or Kyte-Doolittle hydrophilicity values. Peptide structure and Plotstructure provided "antigenic index" plots. The GCG package also includes a program called ANTIGENIC which implements the algorithm of Kolaskar and Tongaonkar, "A semi-empirical method for prediction of antigenic determinants on protein antigens," FEBS Lett., 276:172-4 (1990).

Enclosed as exhibit 1, are printouts of the epitopes, as

predicted by this algorithm, for omp4- omp14.

It was routine for molecular biologists to use algorithms based on local hydrophilicity, etc. to predict the location of epitopes.

The other approach was to systematically synthesize all possible oligopeptide linear epitopes. See Geysen, et al. USP 4,708,871; WO84/03564; J. Immunol. Meth., 102:259-74 (1987); Science, 235:1184-90 (1987), copies enclosed. Typically, the oligopeptides were 6-10 a.a. in length. Based on the teaching at P15, L32, we would expect the skilled worker to construct a library of all possible hexapeptide fragments. This, too, is considered routine experimentation.

While claim 5 further requires that the variants be at least 80% identical to a reference protein, this is not, strictly speaking, necessary for either activity.[1] However it does increase the chances that the variant will (1) preserve at least one conformational epitope, and (2) present more than one linear epitope. (New independent claim 34 omits the 80% limitation, but requires at least two GGAI motifs and at least one chlamydial OMP epitope.)

Also, while we do not concede that the specification need contain any teaching of which positions can be mutated with little risk of loss of biological activity, the specification in fact contains relevant teachings. We direct the Examiner's attention to page 14, line 24 to page 15, line 8:

> Comparison of the DNA sequences from genes encoding Omp4-15 shows that the overall similarity between the individual genes ranges between 43-55%. Comparison of the amino acid sequences of Omp4-15 shows 34-49%

[1] For example, if a chlamydial hexapeptide epitope were conjugated to BSA or KLH, the fusion protein would be likely to elicit a relevant immune response, even though the fusion protein would be less than 10% identical to the native chlamydial protein.

> identity and 53-64% similarity. The homology is generally scattered along the entire length of the deduced amino acids. However, as seen from figure 8 A-J there are some regions in which the homology is more pronounced. This is seen in the repeated sequence where the sequence GGAI is repeated 4-7 times in the genes. It is interesting that the DNA homology is not conserved for the sequences encoding the four amino acids GGAI. This may indicate a functional role of this part of the protein and indicates that the repeated structure did not occur by a duplication of the gene. In addition to the four amino acid repeats GGAI a region from amino acid 400 to 490 has a higher degree of homology than the rest of the protein, with the conserved sequence FYDPI occurring in all sequences. As further indication of similarity in function the amino acid tryptophan (W) is perfectly conserved at 4-6 localizations in the C-terminal part of the protein.

4. New claims 43 and 44

Applicants used the SDS treated, purified outer membrane complex of C. pneumoniae as an immunogen, obtaining the polyclonal antibody PAB-150. See P5, L30-36 and P25, L27-P26, L12. The antiserum recognized OMC proteins with sizes of 100/95, 60 and 38 kDa (P26, L10-12). The specifications notes that "56 proteins according to the invention could be detected in colony blotting of recombinant E. coli" (P6, L2-4).

The PAB 150 polyclonal antibody "contained antibodies to a high number of different epitopes positioned on different members of the protein family", facilitating immunoisolation of the genes. Four were so isolated, and then their sequences were used to design primers by which another eight were isolated. (P6, L25-37).

Applicants generally contemplate use of the surface exposed

outer membrane C. pneumoniae proteins of the 89-101 kDa and 56-57 kDa families. See P8, L5-20, P8, L31-P9, L3, P9, L23-28.

It would not require undue experimentation to use the eight new genes to isolate additional members of this family, which was shown to have 56 members.

New claim 43 recites recognition of an isolated chlamydial OMP by a polyclonal antibody akin to PAB 150, and the molecular weights noted above. Claim 44 adds the presence of at least two GGAI motifs, which is likely related to the function of these OMPs. Like claim 5 and 34, the claims exclude the presence of other chlamydial proteins, thereby distinguishing Melgosa.

Attached hereto is a marked-up version of the changes made to the specification and claims by the current amendment. The attached page is captioned "Version with markings to show changes made".

Respectfully submitted,

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Enclosures
-Drawings
-Jameson and Wolf (1988)
-Hopp and Woods (1981)
-USP 4,708,871
-WO84/03564
-ANTIGENIC printout (Ex. 1)
-Geysen, Journal of Immunological Methods (1987)
-Geysen, Science (1987)

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VERSION WITH MARKINGS TO SHOW CHANGES MADE

In the claims:

Claim 5 has been amended as follows:

5 (twice amended). A non-naturally occurring or isolated protein or peptide which is (i) an isolated protein derived from Chlamydia pneumoniae having the amino acid sequence selected from the group consisting of SEQ ID NO:2, SEQ ID NO:4, SEQ ID NO:6, SEQ ID NO:8, SEQ ID NO:10, SEQ ID NO:12, SEQ ID NO:14, SEQ ID NO:16, SEQ ID NO:18, SEQ ID NO:20, SEQ ID NO:22, and SEQ ID NO:24, said protein being free of any other chlamydial protein, or (ii) a variant protein having an amino acid sequence identity of at least 80% to at least one of said isolated proteins, or (iii) a peptide or protein which consists of an amino acid sequence which is a subsequence, at least 6 amino acids in length, of at least one of said isolated proteins, said variant protein or subsequence comprising at least one epitope of at least one of said isolated proteins.

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